

THAT WHICH IS CLAIMED:

1. A method for detecting a mutant allele of a wheat acetohydroxyacid synthase large subunit (*AHASL*) gene that confers tolerance to imidazolinone herbicides on a wheat plant, said method comprising the steps of:
- 5 (a) obtaining genomic DNA from a wheat plant;
- (b) using said DNA as a template for a PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a nucleotide sequence with a 5' end and a 3' end, wherein said
- 10 nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12 and said nucleotide sequence has a cytidine at the 3' end; and
- (c) detecting the products of said PCR amplification;
- wherein said mutant-allele-specific primer is capable of annealing to a region of
- 15 an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers.
2. The method of claim 1, wherein said DNA has been subjected to a pre-amplification before step (b), said pre-amplification comprising said DNA, polymerase,
- 20 deoxyribonucleotide triphosphates, a forward *AHASL* primer, and a reverse *AHASL* primer, wherein said forward and reverse *AHASL*-gene-specific primers are capable of annealing to regions of an *AHASL* gene that are nested between the annealing sites of said forward and reverse *AHASL* primers.
- 25 3. The method of claim 2, wherein said DNA is digested with exonuclease following said pre-amplification and before step (b).
4. The method of claim 2, wherein said forward *AHASL* primer and said reverse *AHASL* primer are designed to anneal to *AHASL1A*, *AHASL1B*, and *AHASL1D*.
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5. The method of claim 2, wherein said forward *AHASL* primer comprises the nucleotide sequence set forth in SEQ ID NO: 1.

6. The method of claim 2, wherein said reverse *AHASL* primer comprises the
5 nucleotide sequence set forth in SEQ ID NO: 2.

7. The method of claim 1, wherein said mutant-allele-specific primer comprises the nucleotide sequence set forth in SEQ ID NO: 3.

10 8. The method of claim 1, wherein said *AHASL* gene is *AHASL1D*.

9. The method of claim 8, wherein said forward *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 5 and said reverse *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 7.

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10. The method of claim 1, wherein said *AHASL* gene is *AHASL1B*.

11. The method of claim 10, wherein said forward *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 6 and said reverse *AHASL*-gene-specific
20 primer has the sequence set forth in SEQ ID NO: 8.

12. The method of claim 1, wherein said *AHASL* gene is *AHASL1A*.

13. The method of claim 12, wherein said forward *AHASL*-gene-specific
25 primer has the sequence set forth in SEQ ID NO: 5 and said reverse *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 9.

14. The method of claim 1, wherein said detecting comprises gel electrophoresis and ethidium-bromide staining.

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15. A method for analysis of a wheat *AHASL* gene, said method comprising the steps of:

- (a) obtaining genomic DNA from a wheat plant;
 - (b) using said DNA as a template for a first PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a first nucleotide sequence with a 5' end and a 3' end, wherein said first nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12 and said first nucleotide sequence has a cytidine at the 3' end;
 - (c) using said DNA as a template for a second PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, said forward *AHASL*-gene-specific primer, said reverse *AHASL*-gene-specific primer, and a wild-type-allele-specific primer comprising a second nucleotide sequence with a 5' end and a 3' end, wherein said second nucleotide sequence is capable of annealing to the complement of nucleotides 4 to 23 of SEQ ID NO: 10 and said second nucleotide sequence has a guanosine at the 3' end; and
 - (d) detecting the products of said first and said second PCR amplifications;
- wherein said wild-type-allele-specific primer and said mutant-allele-specific primer are capable of annealing to a region of an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers.

16. The method of claim 15, wherein said DNA has been subjected to a pre-amplification before step (b), said pre-amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL* primer, and a reverse *AHASL* primer, wherein said forward and reverse *AHASL*-gene-specific primers are capable of annealing to regions of an *AHASL* gene that are nested between the annealing sites of said forward and reverse *AHASL* primers.

17. The method of claim 16, wherein said DNA is digested with exonuclease following said pre-amplification and before step (b).

18. The method of claim 16, wherein said forward *AHASL* primer and said reverse *AHASL* primer are designed to anneal to *AHASL1A*, *AHASL1B*, and *AHASL1D*.

5 19. The method of claim 16, wherein said forward *AHASL* primer comprises the nucleotide sequence set forth in SEQ ID NO: 1.

20. The method of claim 16, wherein said reverse *AHASL* primer comprises the nucleotide sequence set forth in SEQ ID NO: 2.

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21. The method of claim 15, wherein said mutant-allele-specific primer comprises the nucleotide sequence set forth in SEQ ID NO: 3.

15 22. The method of claim 15, wherein said wild-type-allele-specific primer comprises the nucleotide sequence set forth in SEQ ID NO: 4.

23. The method of claim 15, wherein said *AHASL* gene is *AHASL1D*.

20 24. The method of claim 23, wherein said forward *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 5 and said reverse *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 7.

25. The method of claim 15, wherein said *AHASL* gene is *AHASL1B*.

25 26. The method of claim 25, wherein said forward *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 6 and said reverse *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 8.

27. The method of claim 15, wherein said *AHASL* gene is *AHASL1A*.

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28. The method of claim 27, wherein said forward *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 5 and said reverse *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 9.

5 29. The method of claim 15, wherein said detecting comprises gel electrophoresis and ethidium-bromide staining.

30. A method for analysis of a wheat *AHASL* gene, said method comprising the steps of:

- 10 (a) obtaining genomic DNA from a wheat plant;
- (b) using said DNA as a template in a pre-amplification comprising said DNA, deoxyribonucleotide triphosphates, polymerase, a forward *AHASL* primer, and a reverse *AHASL* primer, so as to produce pre-amplified DNA;
- (c) using said pre-amplified DNA as a template for a first PCR
- 15 amplification comprising said pre-amplified DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a first nucleotide sequence with a 5' end and a 3' end, wherein said first nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12 and said first nucleotide sequence
- 20 has a cytidine at the 3' end;
- (d) using said pre-amplified DNA as a template for a second PCR amplification comprising said pre-amplified DNA, polymerase, deoxyribonucleotide triphosphates, said forward *AHASL*-gene-specific primer, said reverse *AHASL*-gene-specific primer, and a wild-type-allele-specific primer comprising a second nucleotide
- 25 sequence with a 5' end and a 3' end, wherein said second nucleotide sequence is capable of annealing to the complement of nucleotides 4 to 23 of SEQ ID NO: 10 and said second nucleotide sequence has a guanosine at the 3' end; and
- (e) detecting the products of said first and said second PCR amplifications;

wherein said wild-type-allele-specific primer and said mutant-allele-specific primer are capable of annealing to a region of an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers.

5 31. The method of claim 30, wherein said pre-amplified DNA is digested with exonuclease before step (c).

 32. An oligonucleotide primer for PCR amplification of a fragment of an allele of an *AHASL* gene, said primer having the nucleotide sequence set forth in SEQ ID
10 NO: 3 or SEQ ID NO: 4.

 33. A kit for determining whether a wheat plant comprises in its genome a mutant allele of a wheat *AHASL* gene that confers tolerance to imidazolinone herbicides on a wheat plant, said kit comprising:

15 (a) a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a nucleotide sequence with a 5' end and a 3' end, wherein said nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12, said nucleotide sequence has a cytidine at the 3' end, and said mutant-allele-specific primer is capable of annealing to a
20 region of an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers;

 (b) a polymerase enzyme capable of catalyzing the PCR amplification of a first fragment of a wheat *AHASL* gene and a second fragment of a wheat *AHASL* gene, wherein the first fragment is between said annealing site of said forward *AHASL*-
25 gene-specific primer and said annealing site of said reverse *AHASL*-gene-specific primer and the second fragment is between the annealing site of said mutant-allele-specific primer and said annealing site of said reverse *AHASL*-gene-specific primer.

 34. The kit of claim 33, wherein said mutant-allele-specific primer comprises
30 the nucleotide sequence set forth in SEQ ID NO: 3.

35. The kit of claim 33, wherein said *AHASL* gene is *AHASL1D*.

36. The kit of claim 35, wherein said forward *AHASL*-gene-specific primer
has the sequence set forth in SEQ ID NO: 5 and said reverse *AHASL*-gene-specific primer
5 has the sequence set forth in SEQ ID NO: 7.

37. The kit of claim 33, wherein said *AHASL* gene is *AHASL1B*.

38. The kit of claim 37, wherein said forward *AHASL*-gene-specific primer
10 has the sequence set forth in SEQ ID NO: 6 and said reverse *AHASL*-gene-specific primer
has the sequence set forth in SEQ ID NO: 8.

39. The kit of claim 33, wherein said *AHASL* gene is *AHASL1A*.

40. The kit of claim 39, wherein said forward *AHASL*-gene-specific primer
15 has the sequence set forth in SEQ ID NO: 5 and said reverse *AHASL*-gene-specific primer
has the sequence set forth in SEQ ID NO: 9.

41. The kit of claim 33, further comprising a forward *AHASL* primer and a
20 reverse *AHASL* primer.